Molecular Human Reproduction vol.5 no.12 pp. 1155–1161, 1999

# Expression of ut roglobin in th human ndom trium

**Frank Müller-Schöttle[1,3], Irmgard Classen-Linke[1], Joachim Alfer[1], Claudia Krusche[1], Karin Beier-Hellwig[1], Karl Sterzik[2] and Henning M.Beier[1]**

[1]Department of Anatomy and Reproductive Biology, RWTH University of Aachen, Wendlingweg 2, D-52057 Aachen, and [2]Institute for Reproductive Medicine, Frauenstrasse 51, D-89073 Ulm, Germany

[3]To whom correspondence should be addressed

**Ut roglobin is a progesterone binding protein, a member of the antiflammin gene family and possibly a novel cytokine. Initially, uteroglobin was identified as the major protein of rabbit uterine secretion during the phase of preimplantation. Counterparts of the rabbit uteroglobin or its gene are described in rat, mouse, hamster, hare, pig, horse and human. While uteroglobin appears as one of the most extensively studied proteins, particularly its physico–chemical properties, including its crystal structure and its gene, the true physiological role of this protein still remains to be unravelled. Essential to understanding the significance of human uteroglobin in reproductive organs, particularly in the endometrium, is a knowledge of the spatial and chronological expression of this secretory protein. Our studies on 115 volunteers combined reverse transcription–polymerase chain reaction (RT–PCR), immunohistochemistry and quantitative assessment by an nzyme-linked immunosorbent assay for uteroglobin. The expression, localization and release of uteroglobulin in the human endometrium are presented. Secretory uteroglobin is found in endometrial tissue homogenates in highest levels of expression during the early luteal phase (days 15–19, 340 pg/mg total protein). In turn, uteroglobin is released into the uterine lumen in peak amounts during the receptive phase of the menstrual cycle (mid-luteal phase, days 20–23, secretion level 833.4 pg/mg total protein). Our immunohistochemical studies match with these results, as uteroglobin is located during the early and mid-luteal phase in the apical compartments of endometrial gland cells. These observations strongly suggest an involvement of uteroglobin in endometrial preparations for implantation.**

*Key words:* CC10/CC16/human endometrium/menstrual cycle/uteroglobin

## Introduction

Uteroglobin was first described as a steroid-dependent protein in flushed uterine secretion during the preimplantation phase and as a major component of blastocyst fluid in the rabbit (Beier, 1966, 1968). Independently, Krishnan and Daniel described this protein in flushed uterine fluid of the rabbit and coined its name 'blastokinin' (Krishnan and Daniel, 1967). From the beginning, it was identified as an unusually small, globular endometrial secretory protein, which proved to be both progesterone regulated (Beier, 1968; Bullock, 1980; Hegele-Hartung and Beier, 1986) and a progesterone binding protein (Urzua *et al.*, 1971; Beato and Beier, 1978). In the meantime, uteroglobin has become one of the best characterized proteins with regard to its physico–chemical properties, including its crystal structure and molecular biology (for reviews see Beier, 1982; Beato *et al.*, 1989; Miele *et al.*, 1994), but the physiological role of uteroglobin still remains an enigma. The gene coding for uteroglobin was first described in the rabbit (Atger *et al.*, 1980) followed by reports on the hare (Lopez de Haro and Nieto, 1986), human (Singh *et al.*, 1988), rat (Nordlund-Möller *et al.*, 1990), mouse (Ray *et al.*, 1993), hamster (Sagal and Nieto, 1998a) and pig (Sagal and Nieto, 1998b) and have induced numerous investigations on its distribution in various organs and its hormone-regulated expression. Recently, its progesterone-dependent expression could be shown in the equine endometrium (Beier-Hellwig *et al.*, 1995). After its original description in the rabbit uterus, uteroglobin was also found in other organs, particularly in epithelial cells, including those of respiratory, gastrointestinal, and genito–urinary systems of both genders. In the human, uteroglobin equivalent molecules were first described as Clara cell secretory proteins detected in lung lavage (Dhanireddy, 1988). In 1993 it was shown that the rabbit uteroglobin protein and the human Clara cell secretory protein (Clara Cell 10 kDa protein or CC10) are the same (Mantile *et al.*, 1993). Nevertheless, it was postulated and found in other organs, e.g. uterus (Beier, 1978; Cowan *et al.*, 1986; Wolf *et al.*, 1992; Peri *et al.*, 1994), prostate (Manyak *et al.*, 1988) and in body fluids, e.g. urine (Jackson *et al.*, 1988) and blood (Aoki *et al.*, 1996). It was shown that *uteroglobin* mRNA is expressed in a human endometrial tumour cell line and that two weak binding sites for progesterone receptors are partially conserved in the 5′- flanking region of the human *uteroglobin* gene (Wolf *et al.*, 1992). Immunofluorescent localization of uteroglobin was found in the human endometrium (Peri *et al.*, 1994), indicating somewhat higher levels of uteroglobin expression during the luteal

© European Society of Human Reproduction and Embryology

phase. We have described already (Aoki *et al.*, 1996) the presence of uteroglobin in small amounts in blood, and this study focuses on the biological significance of human uteroglobin in reproductive organs, especially the endometrium. An essential prerequisite to start with in those studies, is the exact cellular localization and studies on the expression of this protein. Uteroglobin expression, localization and secretion in the endometrium are now presented. Reverse transcription–polymerase chain reaction (RT–PCR) data demonstrate the expression of uteroglobin in the endometrium, with the highest values in the luteal phase. Using immunohistochemistry, we have also successfully demonstrated the presence of uteroglobin in both the epithelial cells of endometrial glands and the luminal epithelium. Finally, the study was completed by investigating the secretory release of uteroglobin in luminal secretions, using an enzyme-linked immunosorbent assay (ELISA).

## Materials and methods

### *Tissue, secretion and serum samples*

Endometrial tissue samples, secretion and serum samples were obtained from women undergoing diagnostic investigations in an assisted reproduction therapy programme in the Institute for Reproductive Medicine, Ulm, Germany. The male partners alone were the reason for inclusion in the study. Informed consent was obtained from each woman ($n$ = 115), prior to the beginning of diagnostic procedures. Samples of tissue, secretion and sera were only included in the present study if the female partner was healthy and did not show any indication of infertility.

The secretion samples were aspirated by an intra-uterine catheter (Kremer Delafontaine, CCD, Paris, France) using no washing or flushing techniques. Immediately after collecting these secretion samples (40–60 µl), endometrial tissue biopsies were obtained using a Novak currette. Tissues were split for histological assessments and for isolation of total RNA or for tissue homogenization. At the same time, blood samples were taken for processing serum samples of the women. These have been investigated for luteinizing hormone (LH), progesterone, oestradiol, and particularly also for uteroglobin. Dating of all samples according to Noyes criteria has been performed taking into account the endocrine parameters, however, most definitely using the histological assessment. For adjustment, we compared standard preparations from our endometrial tissue bank (Institute of Anatomy and Reproductive Biology, RWTH University of Aachen). Uteroglobin immunohistochemistry, in addition, was compared using paraffin sections of lung tissue samples, containing parts of histologically normal bronchial branches and parenchyme, which had been prepared routinely by the Institute of Pathology (RWTH University of Aachen) in consequence of various pulmonary surgeries.

### *Oliginucleotide primers*

Purified oligonucleotide primers for amplification of human uteroglobin and human cytochrome oxidase subunit I (CYT) cDNA were synthesized by MWG Biotech (Ebersberg, Germany). The sequence of the uteroglobin forward primer was 5′-CTC ACC CTG GTC ACA CTG G-3′ (temperature = 61°C; nucleotides 26–44). The sequence of the uteroglobin reverse primer was 5′-GGG TGG ACT CAA AGC ATG G-3′ (temperature = 59°C; nucleotides 329–345). The sequence of the CYT forward primer was 5′-CGT CAC AGC CCA TGC ATT TG-3′ (temperature = 57°C; nucleotides 271–290). The sequence of the CYT reverse primer was 5′-GGT TAG GTC TAC GGA GGC TC-3′ (temperature = 50°C; nucleotides 519–538).

### *RNA extraction*

Total RNA was extracted from the endometrial samples using the RNAzol™ method (Chomczynski and Sacchi, 1987), according to the manufacturer's instructions (WAK Chemie, Bad Soden, Germany). RNA concentrations were determined spectrophotometrially by absorption at 260 nm.

### *cDNA synthesis*

Reverse transcription and cDNA synthesis of total RNA from endometrial samples were performed using the Biometra Personal Cycler (Göttingen, Germany). All additives (without the oligo dT primer; MWG Biotech, Ebersberg, Germany) used for the mixture for reverse transcription (20 µl/tube) were from MBI Fermentas (Vilnius, Lithuania). Endometrial RNA (1 µg/tube) was heated for 10 min at 65°C and then kept at 4°C for 5 min. After addition of the RNA to the reaction mixture (5 × cDNA synthesis reaction buffer, 1 mM dNTP mixture, 1.6 µg oligo dT primer, 20 units RNase inhibitor, 20 units M-MuLV reverse transcriptase) the samples were reverse-transcribed at 37°C for 1 h.

### *PCR amplification*

PCR amplification of the cDNA from endometrial samples was carried out using the Biometra Personal Cycler (Göttingen, Germany). All additives (without the primers; MWG Biotech) used for the mixture for PCR (50 µl/ tube) were obtained from MBI Fermentas (PCR reaction buffer, 3 mM $MgCl_2$, 0.2 mM dNTP mixture, 0.2 µM primer I and II, 2 units Taq DNA polymerase, 2 µl cDNA, and 50 µl $H_2O$).

For uteroglobin amplification a touch-down protocol was used: 1 min at 94°C, 10 cycles with 30 s at 94°C, 1 min at 60–58°C (i.e. in the first 10 cycles, the annealing temperature was decreased from 60 to 58°C with a 0.2°C temperature decrease per cycle), then 30 s at 72°C, followed by 24 cycles with 30 s at 94°C, 30 s at 58°C, 30 s at 72°C, and 3 min at 72°C. For CYT amplification, a standard protocol was used: 5 min at 92°C, 21 cycles with 1 min at 92°C, 1 min at 57°C, 2 min at 2°C, and 3 min at 72°C.

### *Detection of PCR products*

10 µl of each PCR product was subjected to electrophoresis on 2% agarose gel and stained with ethidium bromide. After electrophoresis, the gel was placed on an UV-transilluminator, processed with gel print 2000i station (MWG Biotech, Ebersberg, Germany) and analysed using ONE-DSCAN (Scanalytics, a division of CSPI, Billerica, MA, USA).

### *Immunohistochemistry*

Immunhistochemistry was performed on human endometrium paraffin sections (4–5 µm) using the Histostain-SP kit (Zymed, San Francisco, USA), which is based on streptavidin–biotin amplification. Paraffin-embedded sections were deparaffinized, rehydrated in a graded series of ethanol. The endogenous peroxidase was blocked for 25 min by methanol/$H_2O_2$ (30%) 9:1. Non-specific binding of the antiserum was blocked for 10 min with swine serum 1:20 in phosphate-buffered saline (PBS)/1.5% bovine serum albumin. For positive reaction a polyclonal uteroglobin antiserum (rabbit anti-human uteroglobin antiserum was kindly provided by Dr Jörg Klug, Marburg, Germany) was incubated at 4°C overnight in a 1:100 dilution. Normal rabbit serum was used as a negative control at the same dilution as the antiserum. After washing in PBS, the sections were incubated with a biotinylated second multilink (Dako, Hamburg, Germany) antibody

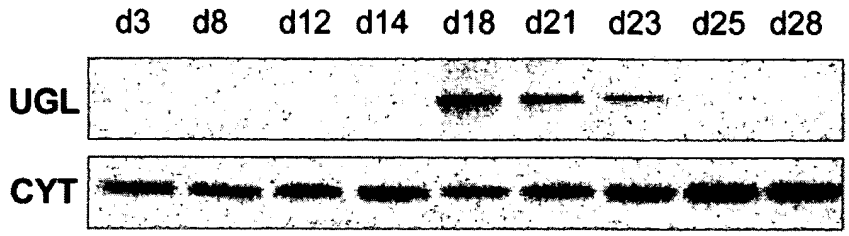


**Figure 1.** Reverse transcription–polymerase chain reaction (RT–PCR) analysis of the expression of human *uteroglobin* mRNA throughout the menstrual cycle. To obtain semiquantitative results for the assessment of *uteroglobin* mRNA, an identical amount of cDNA was used for each PCR with primers which amplify a fragment of the housekeeping gene cytochrome oxidase subunit I (*CYT*). The expression of the *uteroglobin* gene is clearly documented from days 18 to 23 of the menstrual cycle.

for 1 h in a dilution 1:50, which was followed by streptavidin–peroxidase conjugate. Visualizing the specific antigen was performed by peroxidase catalysing the substrate and converting the chromogen AEC (Zymed) to a red deposit.

### *Uteroglobin ELISA*

Analysis of uteroglobin concentrations in secretion, tissue homogenates and in serum was performed by the uteroglobin/CC16-ELISA kit (Eurogenetics, Tessenderlo, Belgium) according to the manufacturer's instructions. Endometrial samples were homogenized in distilled water and centrifuged at 10 600 *g* for 10 min in a Eppendorf 5417 C centrifuge. Endometrial secretions were sonicated and centrifuged to remove cellular debris. The protein concentration of the supernatant of the endometrial samples and secretions was measured by a protein assay (Lowry *et al.*, 1951). 210 µg protein equivalent aliquots of the centrifuged supernatant were lyophilized. After lyophilization, the probes were dissolved in 210 µl distilled water and 125 µl of the prepared probes or 125 µl serum, were analysed using the uteroglobin/CC16-ELISA kit. The minimum detectable concentration of uteroglobin was <50 µg/ml. The inter-assay variability was tested with one sample in 16 tests and was 8.6%. The intra-assay variability was tested with one sample on eight different positions on the same ELISA plate and was 1.6%.

## Results

### *Detection of uteroglobin-specific mRNA in human endometrium*

Total RNA from endometrium of women in different phases of the menstrual cycle were analysed by RT–PCR using uteroglobin-specific primers. Specific bands of the expected size (319 bp) were detected and a typical PCR result is shown in Figure 1. To confirm whether the PCR product obtained was identical with human *uteroglobin* mRNA, the product underwent sequencing analysis at SEQLAB (Göttingen, Germany). The PCR product showed 100% homology with human uteroglobin. To obtain semiquantitative results and for testing the cDNA synthesis, PCR was carried out to detect the *CYT* housekeeping gene (Figure 1). Finally, 80 RNA preparations from endometrial tissue samples, obtained during various phases of the menstrual cycle, were analysed. The menstrual cycle was split into four groups, comprising 20 samples each (follicular phase, days 1–14; early secretory phase, days 15–19; mid-luteal phase, days 20–23; and late luteal phase, days 24–28).

For the PCR with uteroglobin-specific primers, we used the same amount of those cDNA which were first tested using PCR with the CYT-specific primers (Figure 1). Results with the uteroglobin-specific primers indicated that the uteroglobin-specific mRNA was present during the follicular phase, but showed very weak transcription, although increasing levels were found in the early and mid-luteal phases. After assessing the photometric quantification of all the samples tested we found that the cDNA bands increased by a factor of two in the early and mid-luteal phases, compared with the follicular phase. The uteroglobin-specific bands detected during the follicular phase were either very weak (45%) or totally absent (55%), whereas in the early and mid-luteal phases, the majority of samples tested (70%) cDNA gave clear signals. In the late luteal phase, only 25% of the samples tested showed signals and after day 26, we could not detect any *uteroglobin* mRNA.

### *Immunohistochemical detection of uteroglobin in human endometrium*

Paraffin sections from all phases of the menstrual cycle were used to detect uteroglobin antigenicity with a specific polyclonal uteroglobin antibody. As shown in Figure 2, representative results of the uteroglobin immunoreactivity show clear differences between the follicular and luteal phases. As a positive control of uteroglobin-specific antigenicity, we compared paraffin sections from human lung and obtained clear staining of the Clara cells in the bronchiolar branches. Immunohistochemistry of human endometrium sections revealed a higher expression of uteroglobin during the luteal phase (Figure 2c) compared with the follicular phase (Figure 2a). Uteroglobin immunoreactivity was found in both the epithelial cells of the endometrial glands and in the luminal epithelium. The distribution of uteroglobin suggests that it is released via apical extrusion.

### *Analysis of uteroglobin concentrations by ELISA*

Uteroglobin concentrations were measured in serum, homogenates of endometrial tissue and endometrial secretion samples with a specific uteroglobin-ELISA. The data from the ELISA are shown in Figure 3 and Table I. The statistical analysis of the data were carried out using SPSS for Windows, version 7.5. The significance test was based on the Mann–Whitney *U*-test (a non-parametric test).

There was almost no variation in uteroglobin concentration in the serum probes throughout the whole menstrual cycle (47 ng/ml serum; SEM = 6; $n$ = 77). However, in the endometrial tissue homogenates, we found a significant

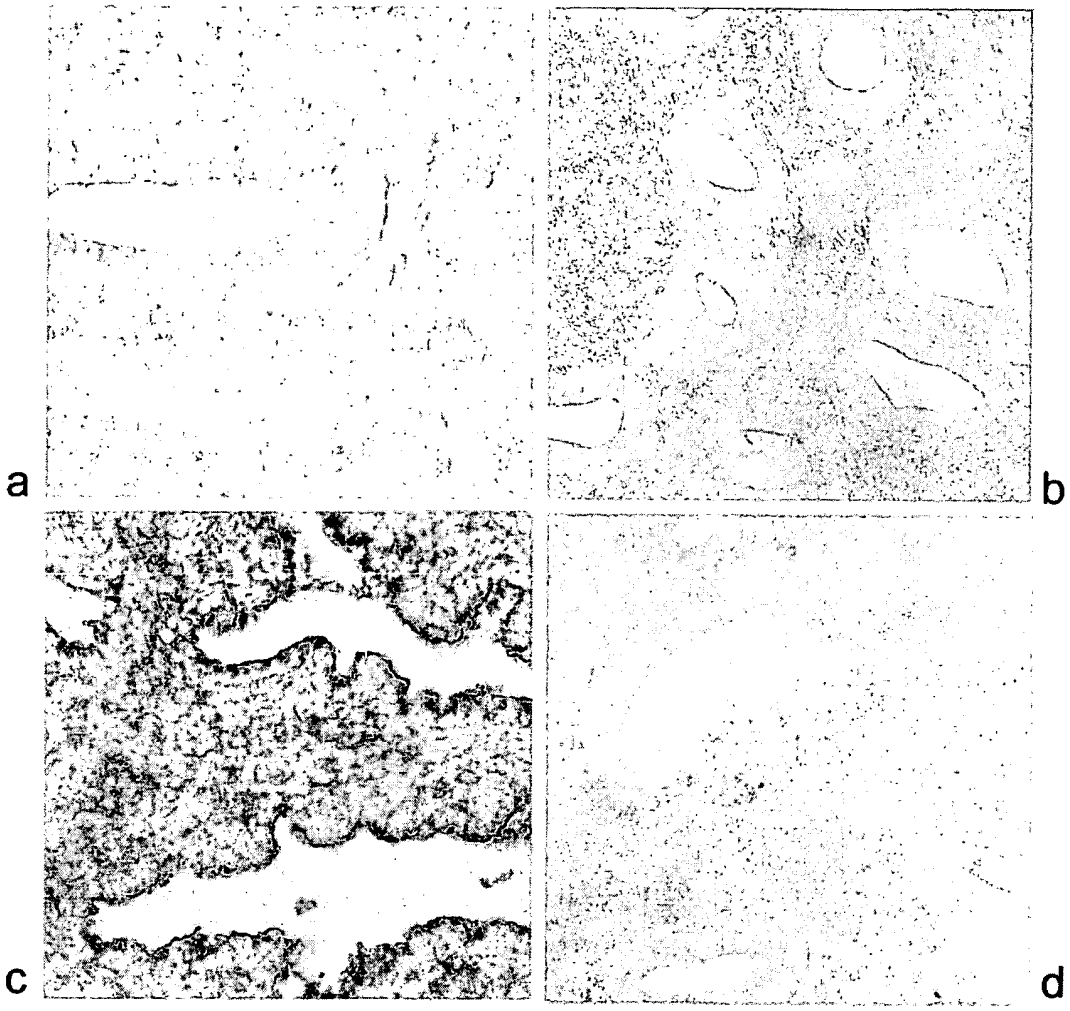


**Figure 2.** Immunohistochemical localization of uteroglobin in the endometrium throughout the menstrual cycle. (**a**, **b**) day 7 of the cycle; (**c**, **d**) day 23 of the cycle. Uteroglobin was detected by a specific polyclonal rabbit antiserum. Strongly positive reactions showed that this protein was localized mainly in the apical compartments of endometrial glands (**c**, day 23). For proof of antibody specificity and demonstration of negative control, the paraffin sections were incubated with normal rabbit serum using the same concentration as the polyclonal antiserum (**b**, day 7; **d**, day 23). Original magnification (**b**, **d**) ×180 and (**a**, **c**) ×360.

increase in uteroglobin concentration in the early luteal ($P$ = 0.004), mid-luteal ($P$ = 0.04) and late luteal phases ($P$ = 0.027), compared with the follicular phase of the menstrual cycle. In endometrial secretion samples, uteroglobin concentrations were significantly increased in the mid-luteal phase compared with the follicular ($P$ = 0.019), early luteal ($P$ = 0.007) and late luteal phase ($P$ = 0.006). Finally, uteroglobin concentrations were always higher in the secretion samples, in comparison with endometrial tissue homogenates. The differences in uteroglobin concentrations in homogenates versus secretion samples were also significant (in the mid-luteal phase, secretion versus homogenates $P$ = 0.003). In conclusion, our ELISA data match well with PCR and immunohistochemistry and confirm the significantly increasing uteroglobin protein expression during the luteal phase compared with the follicular phase. However, remarkably, it is also a significant phenomenon that uteroglobin decreased towards the end of the cycle, indicating a rather unique course of expression in comparison to other marker molecules of endometrial receptivity.

## Discussion

It is now possible to present a consistent and conclusive record on uteroglobin expression throughout the human menstrual cycle. It has been >20 years since the initial observations on immuno-crossreacting 'uteroglobin-like' antigens had been described on episodically collected probes from only a few patients, who underwent hysterectomy (Beier, 1978) or a small number of patients selected for uterine washings (Cowan *et al.*, 1986).

The expression pattern of uteroglobin in the rabbit endometrium during the preimplantation period and in the blastocyst (Beier, 1966, 1968) has been well characterized, together with its progesterone-dependent regulation (Beier, 1968; Bullock, 1980; Hegele-Hartung and Beier, 1986). Most of the studies which deal with human uteroglobin have focused on the lung, where uteroglobin is a product of the Clara cells (CC10/CC16). However, our interest concentrates on the significance of uteroglobin in the human endometrium (Beier and Beier-Hellwig, 1998). One of the first studies of the expression of uteroglobin in the human endometrium (Peri *et al.*, 1994) was

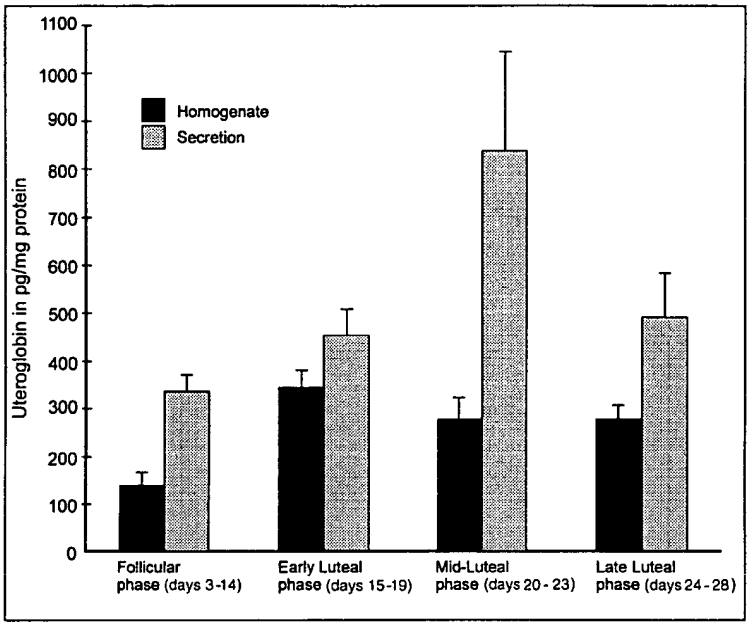


**Figure 3.** Uteroglobin concentrations in endometrial tissue homogenates and endometrial secretion samples measured by enzyme-linked immunosorbent assay (ELISA). The uteroglobin concentrations in secretion were always higher compared with the tissue homogenates and this difference is significant in the mid-luteal phase. There are obviously higher concentrations in samples obtained during the luteal phase. In endometrial tissue homogenates the uteroglobin concentrations were significantly lower in the follicular phase than in the early luteal phase. In endometrial secretion samples, the uteroglobin concentrations were significantly higher in the mid-luteal phase than in the follicular phase, in the early luteal phase or in the late luteal phase.

**Table I.** Uteroglobin protein concentrations in endometrial tissue homogenates and endometrial secretion: statistical assessment data

| Phase of cycle | Endometrial tissue homogenates | | | Endometrial secretion | | |
|---|---|---|---|---|---|---|
| | Average of uteroglobin conc. (pg/mg prot.) | SEM | Samples (*n*) | Average of uteroglobin conc. (pg/mg prot.) | SEM | Samples (*n*) |
| Follicular phase (days 3–14) | 132.5 [a,b,c] | 21.9 | 5 | 332.9 [d] | 32.3 | 5 |
| Early luteal phase (days 15–19) | 340.0 [a] | 33.2 | 8 | 454.0 [e] | 55.0 | 32 |
| Mid-luteal phase (days 20–23) | 281.4 [b] | 39.0 | 12 | 833.4 [d,e,f] | 208.0 | 29 |
| Late luteal phase (d24-d28) | 278.9 [c] | 27.7 | 19 | 492.4 [f] | 85.0 | 32 |

[a,b,c,d,e,f]Significant difference ($P < 0.05$; Mann–Whitney U test) between identical superscripts.

carried out using RT–PCR and immunofluorescence with, however, only a very small number of samples. Uteroglobin expression, localization and secretion in the endometrium are now presented on the investigative basis of 115 healthy volunteers.

Compared with rabbit uteroglobin, human uteroglobin displays some significant similarities and a number of interesting differences in its expression pattern. In early pregnant rabbits, uteroglobin is the major protein in endometrial secretions and is found in mg amounts in one individual uterus. In contrast, human uteroglobin expression is at a considerably lower level, and detection of *uteroglobin* mRNA requires highly sensitive methods, e.g. RT–PCR. Nevertheless, detection of uteroglobin in human blood filtrates (Aoki *et al.*, 1996) has introduced new approaches in investigations of the endometrium. We have clearly demonstrated the expression of uteroglobin by immunohistochemistry (using polyclonal antibodies) and by ELISA (using monoclonal antibodies). The immunohistochemical data indicate that human uteroglobin shows the same localization in human endometrium as rabbit uteroglobin in rabbit endometrium. Uteroglobin is a secretory protein located apically in both the glandular epithelial cells and in the luminal epithelium. In the follicular stages of endometrium, uteroglobin expression was either not detected or was only poorly detected, whereas, in the luteal phase, uteroglobin expression could be detected up to the end of the menstrual cycle.

Using RT–PCR we obtained semiquantitative evidence for the *uteroglobin* mRNA transcription throughout the whole menstrual cycle. On the basis of two biopsy samples, it was estimated that *uteroglobin* mRNA may be transcribed three times more during the luteal phase than during the follicular phase (Peri *et al.*, 1994). This estimation is close to our results, which show the same tendency. However, a more realistic quantification of uteroglobin expression and release is possible when an ELISA is used. In endometrial secretions and in endometrial tissue homogenates we found a significant two-fold increase of uteroglobin expression during the luteal phase, compared with the follicular phase.

The up-regulation of uteroglobin during the luteal phase is of particular interest. On the one hand, it is an interesting endocrinological problem whether this regulation is controlled by progesterone as our results suggest or, on the other hand, it may be rewarding to integrate this phenomenon into the search for uteroglobin function. Wolf *et al.* investigated the genes for possible identification of steroid responsive elements (Wolf *et al.*, 1992). Sequence alignments of the human with the rabbit uteroglobin 5′-flanking regions do not point to an identical regulation by steroids. The well-defined oestrogen responsive element of the rabbit *uteroglobin* gene is not conserved in the human gene. The cluster of progesterone receptor binding sites, as found in the rabbit, however, are partially conserved in the human *uteroglobin* gene. It is an open question, whether the two partially-conserved response elements can still bind the two types of progesterone receptors (PR-A, PR-B) or whether unidentified response elements could do this. In addition, a computer search for potential progesterone/glucocorticoid receptor binding sites revealed five half-palindromic recognition sites (Wolf *et al.*, 1992).

The significance of uteroglobin in the human endometrium has also been investigated by studying uteroglobin expression in consequence of various progestin stimulations using a hormone-responsive primary endometrial cell culture system as described elsewhere (Classen-Linke *et al.*, 1997, 1998).

The relatively low up-regulation of uteroglobin expression (by about a factor of two) in human endometrium as found in the present study, appears to be similar to the uteroglobin induction by corticosteroids in the Clara cells of rabbits (by about a factor of two to two and a half; Savouret *et al.*, 1980; Lombardero and Nieto, 1981). There are now discussions on whether uteroglobin is an immunomodulatory molecule (Miele *et al.*, 1994; Moreno *et al.*, 1997) or whether it is a novel cytokine (Mukherjee *et al.*, 1999). Both theories support the necessity for only a low up-regulation, rather than an excessively increased production as in the rabbit endometrium (>100-fold expression). Recent in-vitro investigations on CC16/uteroglobin synthesis in the lungs of mice indicate a strong likelihood that interferon γ is involved in uteroglobin up-regulation (Magdaleno *et al.*, 1997) and that interferon γ shows a functional inhibition and reduced production in monocytes by CC16/uteroglobin (Dierynck *et al.*, 1995).

Further studies are required to determine whether uteroglobin is a new endometrial cytokine or a new link in the paracrine regulation of endometrial function. Knock-out-experiments have shown, so far, that uteroglobin O/O-mice are fertile (Stripp *et al.*, 1996; Zhang *et al.*, 1997), nevertheless they challenge the search for more detailed investigations on the cellular and molecular level of endometrial differentiation in such uteroglobin-deficient models.

In conclusion, we have demonstrated consistent human uteroglobin expression throughout the menstrual cycle. Significant up-regulation of about two-fold protein production (per mg of total protein) is achieved during the early and mid-luteal phase of the cycle. This result suggests that uteroglobin expression in the human endometrium is also regulated by progesterone (as in other mammalian species), however, molecular, genetic and cell biological proof of this concept needs further clarification.

## Ackn wl dg m nts

This work was supported by the 'START' research programme, RWTH University of Aachen. We thank Dr E.Bosmans, Eurogenetics/Tessenderlo for kindly providing the uteroglobin/CC16 ELISA kit and Dr J.Klug, Institute of Molecular Biology and Tumorbiology, University of Marburg for kindly providing the polyclonal antibodies, and Dr C. Schmitz for collaboration on the statistical analysis. The excellent technical assistance by Bärbel Bonn, Sabine Eisner, Melanie Krauskopf (Ulm) and Rick Kamps is gratefully acknowledged.

## References

Aoki, A., Pasolli, H.A., Raida, M. *et al.* (1996) Isolation of human uteroglobin from blood filtrate. *Mol. Hum. Reprod.*, **2**, 489–497.

Atger, M., Perricaudet, M., Tiollais, P. and Milgrom, E. (1980) Bacterial cloning of the rabbit uteroglobin structural gene. *Biochem. Biophys. Res. Commun.*, **93**, 1082–1088.

Beato, M. and Beier, H.M. (1978) Characteristics of the purified uteroglobin-like protein from rabbit lung. *J. Reprod. Fertil.*, **53**, 305–314.

Beato, M., Arnemann, J., Chalepakis, G. *et al.* (1989) Gene regulation by steroid hormones. *J. Steroid. Biochem.*, **27**, 9–14.

Beier, H.M. (1966) *Das Proteinmilieu im Blutserum, Uterus und in den Blastocysten des Kaninchens vor der Niadtion.* Colloquium Biochemie der Morphogenese, Konstanz, 1966. Deutsche Forschungsgemeinschaft, Bonn, Germany.

Beier, H.M. (1968) Uteroglobin: a hormone sensitive endometrial protein involved in blastocyst development. *Biochim. Biophys. Acta*, **160**, 289–291.

Beier, H.M. (1978) Control of implantation by interference with uteroglobin synthesis, release and utilization. In Ludwig, H. and Tauber, P.F. (ed.), *Human Fertilization.* Georg Thieme Publishers, Stuttgart, Germany, pp. 191–203.

Beier, H.M. (1982) Uteroglobin and other endometrial proteins: Biochemistry and biological significance in beginning pregnancy. In Beier, H.M. and Karlson, P. (eds), *Proteins and Steroids in Early Pregnancy.* Springer-Verlag Berlin Heidelberg New York, USA, pp. 39–71.

Beier, H.M. and Beier-Hellwig, K. (1998) Molecular and cellular aspects of endometrial receptivity. *Hum. Reprod. Update*, **4**, 448–458.

Beier-Hellwig, K., Kremer, H., Bonn, B. *et al.* (1995) Partial sequencing and identification of three proteins from equine uterine secretion regulated by progesterone. *Reprod. Dom. Anim.*, **30**, 295–298.

Bullock, D.W. (1980) Uterine proteins as markers of progesterone action. In Beato, M. (ed.), *Steroid-Induced Proteins.* Developments in Endocrinology. Vol. 8. Elsevier-North Holland, Amsterdam, The Netherlands, pp. 315–318.

Chomczynski, P. and Sacchi, N. (1987) Single step method of RNA isolation by acid guanidinium thiocynate-phenol-chloroform extraction. *Anal. Biochem.*, **162**, 156–159.

Classen-Linke, I., Kusche, M., Knauthe, R. and Beier, H.M. (1997) Establishment of a human endometrial cell culture system and characterization of its polarized hormone responsive epithelial cells. *Cell Tissue Res.*, **287**, 171–185.

Classen-Linke, I., Alfer, J., Hey, S. *et al.* (1998) Marker molecules human endometrial differentiation can be hormonally regulated under in-vitro conditions as in-vivo. *Hum. Reprod. Update*, **4**, 539–549.

Cowan, R.D., North, D.H., Whitworth, N.S. *et al.* (1986) Identification of a uteroglobin-like antigen in human uterine washings. *Fertil. Steril.*, **45**, 820–823.

Dhanireddy, R. (1988) Detection of uteroglobin-like protein in the human neonatal lung. *Biochem. Biophys. Res. Commun.*, **152**, 1447–1454.

Dierynck, I., Bernard, A., Roels, H. and De Ley, M. (1995) Potent inhibition of both human interferon γ production and biologic activity by the Clara cell protein CC16. *Am. J. Resp. Cell. Mol. Biol.*, **12**, 205–210.

Hegele-Hartung, C. and Beier, H.M. (1986) Distribution of uteroglobin in the rabbit endometrium after treatment with anti-progestin (ZK 98.734): an immunohistochemical study. *Hum. Reprod.*, **1**, 497–505.

Jackson, P.J., Turner, R., Keen, J.N. *et al.* (1988) Purification and partial amino acid sequence of human urine protein 1. *J. Chromatog.*, **452**, 359–367.

Krishnan, R.S. and Daniel, J.C. (1967) 'Blastokinin': inducer and regulator of blastocyst development in the rabbit uterus. *Science*, **158**, 490–492.

Lombardero, M. and Nieto, A. (1981) Glucocorticoid and developmental regulation of uteroglobin synthesis in rabbit lung. *Biochem. J.*, **200**, 487–494.

Lopez de Haro, M.S. and Nieto, A. (1986) Nucleotide and derived amino acid sequences of a cDNA coding for pre-uteroglobin from the lung of the hare (*Lepus capensis*). *Biochem. J.*, **235**, 895–898.

Lowry, O.H., Rosenbrough, N.J. and Farr, A.L. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.

Magdaleno, S.M., Wang, G., Jackson, K.J. *et al.* (1997) Interferon γ regulation of Clara cell gene expression: *in vivo* and *in vitro*. *Am. J. Physiol.*, **272**, L1142–L1151.

Mantile, G., Miele, L., Cordella-Miele, Singh G. *et al.* (1993) Human Clara cell 10 kDA protein is the counterpart of rabbit uteroglobin. *J. Biol. Chem.*, **268**, 20343–20351.

Manyak, M.J., Kikukawa, T. and Mukherjee, A.B. (1988) Expression of uteroglobin-like protein in human prostate. *J. Urol.*, **140**, 176–182.

Miele, L., Cordella-Miele, E., Mantile, G. *et al.* (1994) Uteroglobin and uteroglobin-like proteins: the uteroglobin family of proteins. *J. Endocrinol. Invest.*, **17**, 679–692.

Moreno, J. (1997) Antiflammins: endogenous nonapeptides with regulatory effect on inflammation. *Gen. Pharmacol.*, **28**, 23–26.

Mukherjee, A.B., Kundu, G.C., Mantile-Selvaggi, G. *et al.* (1999) Uteroglobin: a novel cytokine? *Cell. Mol. Life. Sci.*, **55**, 771–787.

Nordlund-Moller, L., Andersson, O., Ahlgren, R. *et al.* (1990) Cloning, structure, and expression of a rat binding protein for polychlorinated biphenyls. Homology to the hormonally regulated progesterone-binding protein uteroglobin. *J. Biol. Chem.*, **265**, 12690–12693.

Peri, A., Cowan, B.D., Bhartiya, D. *et al.* (1994) Expression of Clara cell 10 kD gene in the human endometrium and its relationship to ovarian menstrual cycle. *DNA Cell. Biol.*, **13**, 495–503.

Ray, M.K., Magdaleno, S., O'Malley, B.W. and DeMayo, F.J. (1993) Cloning and characterization of the mouse Clara cell specific 10 kDa protein gene: comparison of the 5'-flanking region with the human rat and rabbit gene. *Biochem. Biophys. Res. Commun.*, **197**, 163–171.

Sagal, R.G. and Nieto, A. (1998a) Molecular cloning of the cDNA and the promoter of the hamster uteroglobin/Clara cell 10-kDa gene (UG/CC10): tissue-specific and hormonal regulation. *Arch. Biochem. Biophys*, **350**, 214–222.

Sagal, R.G. and Nieto, A. (1998b) Cloning and sequencing of the cDNA coding for pig pre-uteroglobin/Clara cell 10 kDa protein. *Biochem. Mol. Biol. Int.*, **45**, 205–213.

Savouret, J.-F., Loosfelt, H., Atger, M. and Milgrom, E. (1980) Differential hormonal control of a mRNA in two tissues – Uteroglobin mRNA in the lung and the endometrium. *J. Biol. Chem.*, **255**, 4131–4136.

Singh, G., Katyal, S.L., Brown, W.E. *et al.* (1988) Amino acid and cDNA nucleotide sequences of human Clara cell 10 kDa protein (CC 10): Comparison of structure and function to uteroglobin. *Biochim. Biophys. Acta*, **1039**, 348–355.

Stripp, B.R., Lund, J., Mango, G.W. *et al.* (1996) Clara cell secretory protein: a determinant of PCB bioaccumulation in mammals. *Am. J. Physiol.*, **271**, L656–664.

Urzua, M.A., Stambaugh, R., Flickinger, G. and Mastroianni, L. (1970) Uterine and oviduct fluid protein pattern in the rabbit before and after ovulation. *Fertil. Steril.*, **21**, 860–865.

Wolf, M., Klug, J., Hackenberg, R. *et al.* (1992) Human CC10, the homologue of rabbit uteroglobin: Genomic cloning, chromosomal localization and expression in endometrial cell lines. *Hum. Mol. Genet.*, **1**, 371–378.

Zhang, Z., Kundu, G.C., Yuan, C.J. *et al.* (1997) Severe fibronectin-deposit renal glomerular disease in mice lacking uteroglobin. *Science*, **276**, 1408–1412.

*Received on July 29, 1999; accepted on September 20, 1999*